

Effect of experimental xylitol and fluoride-containing dentifrices on enamel erosion with or without abrasion *in vitro*

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Abstract: This *in vitro* study aimed to analyze the effect of including xylitol into a fluoridated dentifrice to provide protection against enamel erosion with or without abrasion. Bovine enamel specimens were subjected to erosion or erosion plus abrasion (7 days) and the treatment with the following dentifrices: 10% xylitol; 10% xylitol plus 1,030 ppm F (NaF); 1,030 ppm F; and placebo. The erosive challenges were performed 4 times a day (2 min at a time). The specimens were exposed to the slurries of the dentifrices 2 times daily (15 s at a time). Half of the specimens per group were additionally abraded using an electrical toothbrush (F = 1.5 N). Between the challenges, the specimens were remineralized by artificial saliva. Enamel loss was measured profilometrically (μm). The data were statistically analyzed by two-way ANOVA and Bonferroni's post-hoc test ($P < 0.05$). Ten percent xylitol plus F and F dentifrices significantly reduced enamel erosion compared to placebo and xylitol dentifrices. On the other hand, all dentifrices presented a significant potential to protect against enamel erosion plus abrasion compared to placebo, with 10% xylitol plus F showing the best results. Based on this result, the inclusion of 10% xylitol increased the effect of the fluoridated dentifrice against enamel erosion plus

abrasion *in vitro*. *In situ* or clinical studies are needed to confirm the data. (J Oral Sci 53, 163-168, 2011)

Keywords: abrasion; dentifrice; enamel; erosion; fluoride; xylitol.

Introduction

Dental erosion is defined as loss of tooth substance by exogenous or endogenous acids without bacterial involvement. The most important acids are those found in the diet, such as acidic food and drinks (1), and those originating from the stomach, including gastric acids from regurgitation and reflux disorders (2).

The acidic attack leads to irreversible loss of dental hard tissue, which is accompanied by a progressive softening of the surface (3). This softened zone is more susceptible to mechanical forces such as abrasion (4-8), which in turn have little or no effect on sound dental hard tissues (9).

Although the use of fluoridated dentifrice while brushing the teeth has been credited for the reduction of the prevalence of dental caries (10), fluoridated dentifrice has a limited beneficial effect compared to non-fluoridated dentifrices on dental erosion and abrasion, with a reduction of enamel loss of 30% (8,11).

On the other hand, studies have shown that xylitol, a sugar alcohol (non-acidogenic sweetener), when applied in high concentrations, might be able to bond with calcium ions (12), penetrate into the demineralized surfaces (13,14), and

reduce the calcium and phosphate ion diffusion coefficient to the outside of the lesion (13,15). Chunmuang et al. (16) showed the protection of xylitol when added to orange juice, with or without F, against enamel erosion. Amaechi et al. (17) also pointed out that xylitol and F have an additive effect in the reduction of dental erosion by pure orange juice *in vitro*.

Frequent use of fluoridated dentifrices containing xylitol may increase the effects of this sugar, allowing the maintenance of xylitol at an appropriate level in the oral cavity. There is only one study, however, on the effect of xylitol dentifrices on remineralization of artificial enamel caries (18). The use of xylitol as a dentifrice could become a widespread method to prevent dental demineralization compared to other possible xylitol sources such as chewing gum. Additionally, the incorporation of xylitol into fluoridated dentifrices may increase their preventive potential on abrasion of eroded enamel surfaces. However, so far no studies have reported the topical effects of xylitol, with or without F, on enamel erosion and abrasion.

Therefore, the aim of this *in vitro* study was to analyze the effect of adding xylitol to a fluoridated dentifrice to protect teeth against enamel erosion, with or without abrasion.

Material and Methods

Preparation of specimens

Enamel specimens (4 mm × 4 mm × 3 mm) were prepared from the labial surfaces of bovine incisor crowns. The teeth were stored at 4°C in 0.1% buffered thymol solution (pH 7.0) during this phase. The specimens were cut in the middle of the crown's surface using a ISOMET low-speed saw (Buehler Ltd., Lake Bluff, IL, USA) with 2 diamond disks (Extec Corp., Enfield, CT, USA) separated by a 4 mm-thick spacer. The specimens surfaces were ground flat with water-cooled silicon carbide discs (320-, 600-, and 1200-grade papers; Buehler), and polished with felt paper wet with diamond spray (1 µm; Buehler). After polishing, the specimens were cleaned in an ultrasonic device with deionized water for 2 min. Before the experiment, all the specimens were stored in 100% humidity.

Twenty enamel specimens each were allocated by stratified randomization according to their surface hardness (HMV-2000; Shimadzu Corporation, Tokyo, Japan; Knoop diamond, 25 g, 10 s) values (333 ± 18 KHN) into 4 groups corresponding to the following dentifrices: 10% xylitol; 10% xylitol + 1,030 ppm F (NaF); 1,030 ppm F (NaF); and placebo (all produced by Daudt, Brazil; silica, pH 7.0). The company stated that the dentifrices presented similar composition (demineralized water, sodium benzoate,

mallow extract, sucralose, sorbitol, cellulose carboximetyl, glycerol, silica, sodium laurylsulphate, red dye, and flavoring), except for the presence (or lack thereof) of xylitol/F. The abrasivity of the dentifrices was around 14.5-15.5 µm when plex-glass samples were abraded, regardless of the type of dentifrice (The test was conducted according to the ABO 1999 rule, recognized by ISSO 11609 regulation, 1995). In each group, half of the specimens were subdivided into erosion only ($n = 10$) and erosion plus abrasion ($n = 10$).

Prior to the experiment, 2 layers of nail varnish were applied on two-thirds of the surface of each specimen to maintain reference surfaces for determining loss after the experiment.

Erosive and abrasive challenges

All specimens were submitted to a 7-day erosive de- and remineralization cycle. Each day, erosion was performed using freshly opened bottles of regular cola drink (Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.3, 30 ml/specimen, unstirred, 25°C) 4 times for 2 min each.

The specimens were also treated with fresh dentifrice slurries (ratio = 1 dentifrice: 3 water) 2 times daily (15 s, 0.5 ml/specimen, unstirred, 25°C), after both the first and the last erosive challenges (19). Additionally, during the treatment, half of the specimens in each group were abraded, using an electrical toothbrush (Colgate Motions Multi-action, Brazil) for 15 s (166 oscillations/s) (11,20) (Fig. 1).

The toothbrushes were fixed in a constructed device that allowed the heads of the toothbrushes to be aligned parallel to the specimen surface. The toothbrush head was weighed by a precision scale (Pesola, Switzerland) and the weight converted to power (1 kg = 9.80665 N, F = 1.5 N) (21). The toothbrush heads were replaced daily.

Between erosion, treatment and abrasion, the specimens were rinsed in water for 5 s before being immersed in artificial saliva. The specimens were exposed to artificial saliva (pH 6.8, 30 ml/specimen, unstirred, 25°C) for 2 h between each erosive challenge. After the last daily challenge, the specimens were also stored in artificial saliva overnight. The artificial saliva was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl₂ · 2H₂O, 3 mM NH₄Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K₂HPO₄, 3.3 mM urea, 2.4 mM NaH₂PO₄ and ascorbic acid (pH 6.8) (22) (Fig. 1).

Profilometric measurement

Enamel loss (µm) was quantitatively determined by contact profilometry (Hommel Tester T1000, VS, Schwenningen, Germany), which presents an accuracy

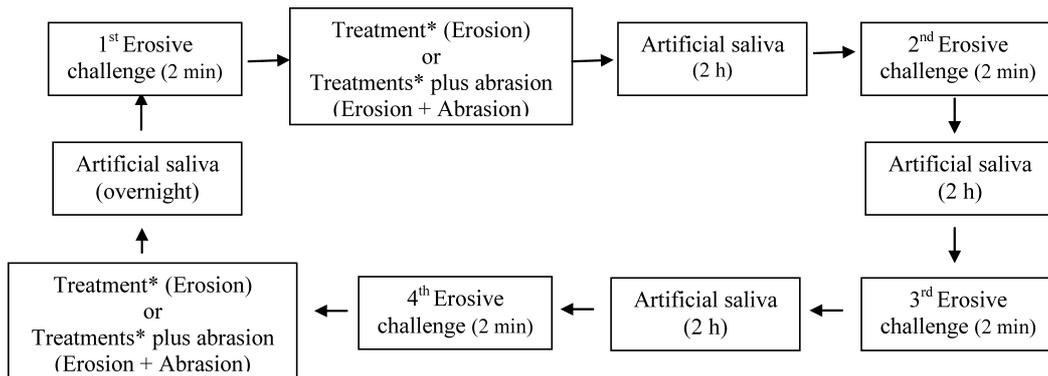


Fig. 1 The daily *in vitro* experiment that was performed for 7 days.

*Treatment with the dentifrice slurries (15 s).

Table 1 Mean enamel loss (μm) \pm SD (95% CI) of specimens subjected to erosion or erosion plus abrasion in the presence of different toothpaste slurries

Treatment	Erosion	Erosion+Abrasion
Placebo	5.1 \pm 0.6 ^a (4.7–5.5)	7.3 \pm 0.9 ^{a*} (6.6–7.9)
10% xylitol	4.9 \pm 1.1 ^a (4.1–5.6)	4.9 \pm 1.2 ^b (4.1–5.8)
10% xylitol + F	3.1 \pm 0.8 ^b (2.5–3.7)	3.9 \pm 1.1 ^c (3.1–4.6)
F	3.8 \pm 0.5 ^b (3.4–4.2)	4.6 \pm 0.8 ^{b*} (4.1–5.2)

Different lower-case superscript letters show significant differences between the toothpastes for each condition.

*indicates significant difference between erosion and erosion plus abrasion for the same toothpaste ($n = 10$, two-way ANOVA, $P < 0.0001$).

around 0.5 μm . For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water) and the specimens were dried (23). The diamond stylus was moved from the first reference to the exposed area and then to the other reference area (Lc = 2.5 mm length). Three profile measurements were randomly performed in the center of each specimen. The vertical distance between the midpoints of regression lines on the reference and experimental areas was defined as tissue loss, and was determined using the software of the device (Hommel tester T 1000). The values were averaged (μm) and submitted for statistical analysis. The standard deviation of repeated analysis of a given sample was 0.4 μm .

Statistical analysis

GraphPad InStat version 2.0 for Windows and GraphPad Prism software version 4.0 for Windows (Graph Pad

Software; San Diego, CA, USA) were used for analysis. The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. Since the assumptions were satisfied, two-way ANOVA and Bonferroni's post-hoc tests were used. The significance level was set at 0.05.

Results

A sample size of 10 specimens was calculated considering an α -error level of 5% and β -error level of 20% (www.ddsresearch.com). Two-way ANOVA revealed a significant difference between the conditions erosion and erosion plus abrasion ($P = 0.0004$) as well as among the dentifrices ($P < 0.0001$). The interaction between the factors was significant ($P = 0.008$). The data in Table 1 shows that 10% xylitol plus F and F dentifrices significantly reduced enamel erosion compared to the placebo and

xylitol dentifrices. On the other hand, all dentifrices presented a significant potential to protect teeth against enamel erosion plus abrasion compared to placebo. Ten percent xylitol plus F produced a significantly better effect than xylitol or F alone on enamel erosion plus abrasion. Eroded and abraded specimens showed a higher enamel loss when compared to eroded specimens for the placebo and F dentifrices.

Discussion

The findings of the present study demonstrated that 10% xylitol plus F dentifrice was similar to F dentifrice in reducing enamel erosion, showing no additive effect of xylitol in this case. However, 10% xylitol plus F produced a better effect on enamel erosion plus abrasion than F dentifrice alone. Since it is assumed that the use of dentifrice is related to toothbrushing, this positive result may allow the development of new preventive strategy for dental erosion plus abrasion. Sano et al. (18) also suggested that inclusion of 5% xylitol in fluoridated dentifrice (500 ppm F) might be useful to enhance enamel caries remineralization compared to using fluoridated dentifrice only.

It is widely believed that the potential of conventional fluoride (such as NaF) to prevent dental erosive demineralization is mainly related to the formation of a calcium fluoride (CaF_2) layer. This is assumed to act as a physical barrier, hampering the contact of the acid with the underlying enamel, or as a mineral reservoir, which is attacked by the erosive challenge, thus promoting remineralization (24, 25). As the remaining demineralized layer of eroded enamel is considerably small compared to the bulk enamel loss, fluoride application predominately aims to prevent further erosive tissue loss rather than to remineralize softened enamel.

On the other hand, it was thought that the action of xylitol in the present study might be related to the fact that this sugar is also able to penetrate into the demineralized enamel surface and form a bond with calcium (13, 15), reducing further enamel demineralization by the erosive challenges. However, this assumption was rejected, since 10% xylitol dentifrice alone was unable to reduce the enamel erosion in the present study.

There are only a few studies of xylitol and dental erosion (16, 17, 26). Our research group recently showed that 10-20% xylitol varnishes and 20% xylitol solutions could significantly reduce the bovine enamel erosion compared to the control group after an experimental period of 5 days (26). After reapplication and more than 5 days of erosive challenges, both xylitol varnishes and solutions significantly reduced the enamel erosion when compared with the control. However, 10% xylitol solution produced

a smooth layer on eroded enamel and significantly reduced the enamel erosion when compared to the placebo varnish/control.

Chunmuang et al. (16) found that the application of 40% xylitol with or without F (227 ppm) solution between the erosive challenges and remineralization was also unable to significantly reduce the human enamel erosion depth and surface softening after an experimental period of 14 days. They reported better results when 25% xylitol with or without F (1 ppm) was added to a beverage. However, Amaechi et al. (17) only showed a preventive effect against enamel erosion when 25% xylitol and 0.5 ppm F were both added to a beverage. The difference in findings might be highly related to the different experimental protocol. Therefore, further studies are needed to clarify this issue.

Regarding dental caries, the addition of xylitol into a remineralizing solution was also able to facilitate calcium movement and accessibility (Ca^{+2} ion carrier) into the pores of artificial caries lesions, inducing remineralization of deeper layers of demineralized enamel in an abiotic model (14). However, it is also important to point out that artificial caries present a subsurface lesion with a less demineralized surface layer, compared to erosion-like lesions (softening surfaces) (27). The type of lesion involved also might explain the different findings. Therefore, the ineffectiveness of xylitol on erosion in the present study might be related to the fact that the demineralized layer of eroded enamel is considerably small.

In this sense, the positive effect of a xylitol dentifrice – regardless of the presence or absence of F – on enamel erosion plus abrasion might not be explained by the known mechanism of action (13,15). We speculate that the effect of xylitol in the present study was related to the abrasive procedure. Xylitol in the toothpaste might act as a lubricant, reducing the impact of brushing on the eroded enamel; even the dentifrices presented the same basic composition and the assay applied by the Company did not show any significant abrasive effect among the dentifrices. The abrasive test performed by the Company was applied on plex-glass samples, which might not reflect what happens on the eroded enamel surface. Accordingly, only the placebo and F dentifrices induced a higher enamel loss when erosion was associated with abrasion compared to erosion alone. For the other treatments, the abrasive procedure did not significantly increase the loss of eroded enamel, which in turn might be explained by the presence of xylitol.

Based on the results, the inclusion of 10% xylitol increases the effect of the fluoridated dentifrice against enamel erosion plus abrasion. Before clinical use, it is advisable to perform *in situ* studies to test the effects of

these new products on human enamel and dentin erosion and abrasion. Furthermore, the quality of eroded enamel treated with the experimental dentifrices should be assessed using SEM in further studies.

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